CLAIMS

1) Isolated single- or double-stranded DNA sequence, represented in Figure 2 (direct or complementary sequence of SEQ ID No.1) corresponding to the *ery*G-*ery*AIII region of the cluster of erythromycin biosynthesis genes.

2) ~~DNA sequence according to claim 1 comprising:~~ A DNA of Claim 42 comprising

- the *ery*BII sequence corresponding to ORF7 (complementary sequence of SEQ ID No.1 from nucleotide 48 to nucleotide 1046) and coding for a dTDP-4-keto-L-6-desoxyhexose 2,3-reductase,
- the *ery*CIII sequence corresponding to ORF8 (complementary sequence of SEQ ID No.1 from nucleotide 1046 to nucleotide 2308) and coding for a desosaminyltransferase and
- the *ery*CII sequence corresponding to ORF9 (complementary sequence of SEQ ID No.1 from nucleotide 2322 to nucleotide 3404) and coding for a dTDP-4-keto-D-6-desoxyhexose 3,4-isomerase.

3) ~~Isolated DNA sequence represented in Figure 2 chosen from~~ the *ery*BII sequence corresponding to ORF7 (complementary sequence of SEQ ID No.1 from nucleotide 48 to nucleotide 1046), the *ery*CIII sequence corresponding to ORF8 (complementary sequence of SEQ ID No.1 from nucleotide 1046 to nucleotide 2308) or the *ery*CII sequence corresponding to ORF9 (complementary sequence of SEQ ID No.1 from nucleotide 2322 to nucleotide 3404) and the sequences which hybridize and/or display significant homologies with this sequence or fragments of the latter and having the same function.

4) ~~Isolated DNA sequence eryCIII represented in Figure 2~~ An isolated DNA sequence eryCIII corresponding to ORF8 (complementary sequence of SEQ ID No.1 from nucleotide 1046 to nucleotide 2308 = complementary sequence of SEQ ID No.4) and coding for a desosaminyl transferase.

5) Polypeptide coded by one of the DNA sequences according to ~~one of claims 1 to 4~~ Claim 1 Claim 42.

6) ~~Polypeptide according to claim 5 corresponding to an ORF represented in Figure 2, chosen from ORF7 (having the~~ sequence of SEQ ID No.2), ORF8 (having the sequence of SEQ ID

No.5) or ORF9 (having the sequence of SEQ ID No.3) and the analogues of this polypeptide.

7) Polypeptide according to claim 5 corresponding to ORF8 represented in Figure 2 (having the sequence of SEQ ID No.5) and having a desosaminyltransferase activity, called EryCIII.

8) Isolated DNA sequence represented in Figure 3 (sequence of SEQ ID No.6) corresponding to the *ery*AI-*ery*K region of the cluster of erythromycin biosynthesis genes.

9) DNA sequence according to claim 8 comprising:

- the *ery*BIV sequence corresponding to ORF13 (sequence of SEQ ID No.6 from nucleotide 242 to nucleotide 1207) and coding for a dTDP-4-keto-L-6-desoxyhexose 4-reductase,
- the *ery*BV sequence corresponding to ORF14 (sequence of SEQ ID No.6 from nucleotide 1210 to nucleotide 2454) and coding for a mycarosyltransferase,
- the *ery*CVI sequence corresponding to ORF15 (sequence of SEQ ID No.6 from nucleotide 2510 to nucleotide 3220) and coding for a dTDP-D-6-desoxyhexose 3-N-methyltransferase,
- the *ery*BVI sequence corresponding to ORF16 (sequence of SEQ ID No.6 from nucleotide 3308 to nucleotide 4837) and coding for a dTDP-4-keto-L-6-desoxyhexose 2,3-deshydratase,
- the *ery*CIV sequence corresponding to ORF17 (sequence of SEQ ID No.6 from nucleotide 4837 to nucleotide 6039) and coding for a dTDP-D-6-desoxyhexose 3,4-deshydratase,
- the *ery*CV sequence corresponding to ORF18 (sequence of SEQ ID No.6 from nucleotide 6080 to nucleotide 7546) and coding for a dTDP-D-4,6-didesoxyhexose 3,4-reductase and
- the *ery*BVII sequence corresponding to ORF19 (sequence of SEQ ID No.6 from nucleotide 7578 to nucleotide 8156) and coding for a dTDP-4-keto-D-6-desoxyhexose 3,5 epimerase.

10) Isolated DNA sequence represented in Figure 3 chosen from the *ery*BIV sequence corresponding to ORF13 (sequence of SEQ ID No.6 from nucleotide 242 to nucleotide 1207), the *ery*BV sequence corresponding to ORF14 (sequence of SEQ ID No.6 from nucleotide 1210 to nucleotide 2454), the *ery*CVI sequence corresponding to ORF15 (sequence of SEQ ID No.6 from nucleotide 2510 to nucleotide 3220), the *ery*BVI sequence corresponding to ORF16 (sequence of SEQ ID No.6 from

nucleotide 3308 to nucleotide 4837), the *ery*CIV sequence corresponding to ORF17 (sequence of SEQ ID No.6 from nucleotide 4837 to nucleotide 6039), the *ery*CV sequence corresponding to ORF18 (sequence of SEQ ID No.6 from nucleotide 6080 to nucleotide 7546) or the *ery*BVII sequence corresponding to ORF19 (sequence of SEQ ID No.6 from nucleotide 7578 to nucleotide 8156) and the sequences which hybridize and/or display significant homologies with this sequence or fragments of the latter and having the same function.

11) Isolated DNA sequence *ery*BV represented in Figure 3 corresponding to ORF14 (sequence of SEQ ID No.6 from nucleotide 1210 to nucleotide 2454) and coding for a mycarosyltransferase.

12) Polypeptide coded by one of the DNA sequences according to ~~one of claims 8 to 11~~ Claim 8.

13) Polypeptide according to claim 12 corresponding to an ORF represented in Figure 3, chosen from ORF13 (having the sequence of SEQ ID No.7), ORF14 (having the sequence of SEQ ID No.8), ORF15 (having the sequence of SEQ ID No.9), ORF16 (having the sequence of SEQ ID No.10), ORF17 (having the sequence of SEQ ID No.14), ORF18 (having the sequence of SEQ ID No.11) or ORF19 (having the sequence of SEQ ID No.12) and the analogues of this peptide.

14) Polypeptide according to claim 12 corresponding to ORF14 represented in Figure 3 (having the sequence of SEQ ID No.8) and having an mycarosyltransferase activity, called EryBV.

15) Use of at least one of the DNA sequences chosen from the sequences *ery*BII (complementary sequence of SEQ ID No.1 from nucleotide 48 to nucleotide 1046), *ery*CIII (complementary sequence of SEQ ID No.1 from nucleotide 1046 to nucleotide 2308) or *ery*CII (complementary sequence of SEQ ID No.1 from nucleotide 2322 to nucleotide 3404) represented in Figure 2, *ery*BIV (sequence of SEQ ID No.6 from nucleotide 242 to nucleotide 1207), *ery*BV (sequence of SEQ ID No.6 from nucleotide 1210 to nucleotide 2454), *ery*CVI (sequence of SEQ ID No.6 from nucleotide 2510 to nucleotide 3220), *ery*BVI (sequence of SEQ ID No.6 from nucleotide 3308 to nucleotide

4837), *ery*CIV (sequence of SEQ ID No.6 from nucleotide 4837 to nucleotide 6039), *ery*CV (sequence of SEQ ID No.6 from nucleotide 6080 to nucleotide 7546) or *ery*BVII (sequence of SEQ ID No.6 from nucleotide 7578 to nucleotide 8156) represented in Figure 3, to synthesize hybrid secondary metabolites in *Sac. erythraea*.

16) Use of at least one of the DNA sequences chosen from the sequences *ery*BII (complementary sequence of SEQ ID No.1 from nucleotide 48 to nucleotide 1046), *ery*CIII (complementary sequence of SEQ ID No.1 from nucleotide 1046 to nucleotide 2308) or *ery*CII (complementary sequence of SEQ ID No.1 from nucleotide 2322 to nucleotide 3404) represented in Figure 2, *ery*BIV (sequence of SEQ ID No.6 from nucleotide 242 to nucleotide 1207), *ery*BV (sequence of SEQ ID No.6 from nucleotide 1210 to nucleotide 2454), *ery*CVI (sequence of SEQ ID No.6 from nucleotide 2510 to nucleotide 3220), *ebry*BVI (sequence of SEQ ID No.6 from nucleotide 3308 to nucleotide 4837), *ery*CIV (sequence of SEQ ID No.6 from nucleotide 4837 to nucleotide 6039), *ery*CV (sequence of SEQ ID No.6 from nucleotide 6080 to nucleotide 7546) or *ery*BVII (sequence of SEQ ID No.6 from nucleotide 7578 to nucleotide 8156) represented in Figure 3 or of a fragment of this sequence, as hybridization probes.

17) Use of the *ery*CIII DNA sequence represented in Figure 2 (complementary sequence of SEQ ID No.1 from nucleotide 1046 to nucleotide 2308 = complementary sequence of SEQ ID No.4) as a hybridization probe to isolate genes responsible for the glycosylation of the macrolactone in a macrolide-producing strain.

18) Use according to claim 17 in which the homologous genes are the oleandomycin biosynthesis genes in *S. antibioticus*.

19) Isolated DNA sequence represented in Figure 22 (sequence of SEQ ID No.15) corresponding to a region of the cluster of oleandomycin biosynthesis genes comprising:

- the sequence corresponding to ORF *ole*P1 from nucleotide 184 to nucleotide 1386,
- the sequence corresponding to ORF *ole*G1 from nucleotide 1437 to nucleotide 2714 coding for a glycosyltransferase

activity,
- the sequence corresponding to ORF *ole*G2 from nucleotide 2722 to nucleotide 3999 coding for a glycosyltransferase activity,
- the sequence corresponding to ORF *ole*M from nucleotide 3992 to nucleotide 4720 (= sequence of SEQ ID No.20) and
- the sequence corresponding to ORF *ole*Y from nucleotide 4810 to nucleotide 5967.

20) Isolated DNA sequence represented in Figure 22 chosen from the sequence corresponding to ORF *ole*G1 (sequence of SEQ ID No.15 from nucleotide 1437 to nucleotide 2714 coding for a glycosyltransferase activity and the sequence corresponding to ORF *ole*G2 (sequence of SEQ ID No.15 from nucleotide 2722 to nucleotide 3999) coding for a glycosyltransferase activity.

21) Isolated DNA sequence according to claim 20 corresponding to ORF *ole*G1 (sequence of SEQ ID No.15 from nucleotide 1437 to nucleotide 2714) coding for a desosaminyltransferase activity.

22) Isolated DNA sequence according to claim 20 corresponding to ORF *ole*G2 (sequence of SEQ ID No.15 from nucleotide 2722 to nucleotide 3999) coding for an oleandrosyltransferase activity.

23) Polypeptide coded by the DNA sequence corresponding to ORF *ole*G1 and having a desosaminyltransferase activity (sequence of SEQ ID No.17).

24) Polypeptide coded by the DNA sequence corresponding to ORF *ole*G2 and having an oleandrosyltransferase activity (sequence of SEQ ID No.18).

25) Process for the preparation of hybrid secondary metabolites in *Sac. erythraea* in which:
- a DNA sequence is isolated containing at least one *ery*B sequence or one *ery*C sequence of the cluster of erythromycin biosynthesis genes represented in Figure 2 (complementary sequence of SEQ ID No.1) or in Figure 3 (sequence of SEQ ID No.6),
- a modification is created in said sequence and an altered sequence is obtained,

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- the altered sequence is integrated into the chromosome of the host strain and a modified strain is obtained,
- the modified strain is cultured under conditions which allow the formation of the hybrid secondary metabolite and
- the hybrid secondary metabolite is isolated.

26) Process according to claim 25 in which the DNA sequence codes for one of the enzymes chosen from a
- dTDP-4-keto-L-6-desoxyhexose 2,3-reductase,
- desosaminyltransferase,
- dTDP-4-keto-D-6-desoxyhexose 3,4-isomerase,
- dTDP-4-keto-L-6-desoxyhexose 4-reductase,
- mycarosyltransferase,
- dTDP-D-6-desoxyhexose 3-N-methyltransferase,
- dTDP-4-keto-L-6-desoxyhexose 2,3-deshydratase,
- dTDP-D-6-desoxyhexose 3,4-deshydratase,
- dTDP-D-4,6-didesoxyhexose 3,4-reductase or
- dTDP-4-keto-D-6-desoxyhexose 3,5 epimerase.

27) Process according to claim 25 in which the alteration of the sequence results in the inactivation of at least one of the enzymes chosen from a
- dTDP-4-keto-L-6-desoxyhexose 2,3-reductase,
- desosaminyltransferase,
- dTDP-4-keto-D-6-desoxyhexose 3,4-isomerase,
- dTDP-4-keto-L-6-desoxyhexose 4-reductase,
- mycarosyltransferase,
- dTDP-D-6-desoxyhexose 3-N-methyltransferase,
- dTDP-4-keto-L-6-desoxyhexose 2,3-deshydratase,
- dTDP-D-6-desoxyhexose 3,4-deshydratase,
- dTDP-D-4,6-didesoxyhexose 3,4-reductase or
- dTDP-4-keto-D-6-desoxyhexose 3,5 epimerase.

28) Process according to claim 27 in which the inactivated enzyme is a dTDP-4-keto-L-6-desoxyhexose 4-reductase.

29) Process according to claim 27 in which the inactivated enzyme is a dTDP-D-6-desoxyhexose 3,4-deshydratase.

30) Process according to claim 27 in which the inactivated enzyme is a mycarosyltransferase.

31) Process according to claim 27 in which the inactivated enzyme is a dTDP-4-keto-L-6-desoxyhexose 2,3-reductase.

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32) Process according to claim 25 in which the isolated hybrid secondary metabolite is an analogue of erythromycin chosen from 4"-keto-erythromycin, 4'-hydroxy-erythromycin or 3"-C-desmethyl-2",3"-ene-erythromycin.

33) Process according to claim 25 in which the isolated hybrid secondary metabolite is desosaminyl erythronolide B.

34) Modified strain of *Sac. erythraea* in which at least one of the enzymes chosen from a

- dTDP-4-keto-L-6-desoxyhexose 2,3-reductase,
- desosaminyltransferase,
- dTDP-4-keto-D-6-desoxyhexose 3,4-isomerase,
- dTDP-4-keto-L-6-desoxyhexose 4-reductase,
- mycarosyltransferase,
- dTDP-D-6-desoxyhexose 3-N-methyltransferase,
- dTDP-4-keto-L-6-desoxyhexose 2,3-deshydratase,
- dTDP-D-6-desoxyhexose 3,4-deshydratase,
- dTDP-D-4,6-didesoxyhexose 3,4-reductase or
- dTDP-4-keto-D-6-desoxyhexose 3,5 epimerase

is inactivated and producing at least one hybrid secondary metabolite.

35) Modified strain of *Sac. erythraea* (BII92) in which a dTDP-4-keto-L-6-desoxyhexose 2,3-reductase is inactivated and producing 3"-C-desmethyl-2",3"-ene-erythromycin C.

36) Modified strain of *Sac. erythraea* (BIV87) in which a dTDP-4-keto-L-6-desoxyhexose 4-reductase is inactivated and producing 4"-keto-erythromycin.

37) Modified strain of *Sac. erythraea* (CIV89) in which a dTDP-D-6-desoxyhexose 3,4-deshydratase is inactivated and producing 4'-hydroxyerythromycin D.

38) Modified strain of *Sac. erythraea* (BV88) in which a mycarosyltransferase is inactivated and producing desoaminyl erythronolide B.

39) Preparation process for precursors of oleandomycin in *S. antibioticus* in which

- an alteration is created in the sequence of the gene chosen from the DNA sequence corresponding to ORF *ole*G1 (sequence of SEQ ID No.15 from nucleotide 1437 to nucleotide 2714) and the DNA sequence corresponding to ORF *ole*G2 (sequence of

SEQ ID No.15 from nucleotide 2722 to nucleotide 3999) in the chromosome of a host strain and a modified strain is obtained,

- the modified strain is cultured under conditions allowing the accumulation of the precursors of oleandomycin and
- these precursors are isolated.

40) Process according to claim 39 in which the alteration is created in the DNA sequence corresponding to ORF *ole*G1 (sequence of SEQ ID No.15 from nucleotide 1437 to nucleotide 2714) and the result of which is at least the elimination of the desoaminyltransferase activity and the accumulation of the precursor of oleandomycin 8,8a-desoxyoleandolide.

41) Thymidine 5'-(trihydrogen diphosphate),P'-[3.4,6-tridesoxy-3-(dimethylamino)-D-.xylo.-hexopyranosyl] ester (dTDP-D-desosamine) and the addition salts with bases.

add C1 add C4

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